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(54) Title: STERILIZED 5-AMINOLEVULINIC ACID

(57) Abstract

Colored 5-aminolevulinic acid crystals useful for photodynamic therapy are disclosed. Preferably the colored 5-aminolevulinic acid crystals have the color imparted by irradiation of the crystals, such as gamma radiation. The colored ALA crystals are preferably pharmaceutically pure and sterile and can be contained in a sealed sterile container. Also disclosed is sterile aqueous ALA solution which includes the colored ALA crystals contained in water. Also disclosed is a method for preparing colored ALA crystals which includes exposing non-irradiated ALA crystals to a radiation source at a dose sufficient to impart a color which is different than any color present in the non-irradiated crystals. Preferably the irradiation is sufficient to sterilize the ALA crystals. The sterile colored ALA crystals can be used in a kit for internal or external treatment and/or detection of a condition in a mammal, which includes the sterile, colored ALA crystals and sterile diluent, and in the case of internal treatment and/or detection optionally a catheter for administration of the ALA.

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STERILIZED 5-AMINOLEVULINIC ACID

Background of the Invention

1. Field of the Invention

This invention relates generally to the field of medicine, particularly pharmacotherapeutics and pharmacodetection, using photosensitizing agents and precursors thereof, especially 5-aminolevulinic acid, also known by the acronym "ALA." More specifically, this invention relates to colored ALA, especially sterilized ALA, which is stable under commercial conditions.

2. Description of Related Art

Photodynamic therapy involves the administration of a photosensitizing agent to a subject, including administration of a precursor of a photosensitizing agent such as ALA, and subsequent irradiation with light of the target cells or tissue of the subject. The photosensitizing agent preferentially accumulates in the target cells, namely cells or tissues that are more rapidly proliferating or growing than other cells or tissues in the target environment. The target cells may be more rapidly proliferating because they are malignant or non-malignant, of infective agent origin, e.g. viral, bacteria, parasite or fungal origin or not of infective agent origin; are normally hyperproliferative, such as the endometrium of pre-menopausal women, or are abnormally hyperproliferative, such as cells infected with an infective agent.

Although not intending to be bound by any particularly theory, it has been proposed that following administration of ALA, as a result of their more rapid proliferation, the target cells or tissue contain relatively greater concentrations of light sensitive porphyrins and thus are more sensitive to light.

The target cells or tissue containing sufficiently high concentrations of the photosensitizing agent, including the metabolites of ALA, selectively absorb

greater amounts of light and can be selectively localized and distinguished from the adjacent cells or tissues via fluorescence, or damaged or destroyed. The effect of the light, as is well known in the art, depends on the photosensitizer selected; the wavelength, intensity and duration of administration of the light; and the timing of irradiation vis-a-vis the administration of the photosensitizing agent, and results in fluorescence or impairment or destruction of the target cells or tissue.

A variety of photosensitizing agents have been used for photodynamic therapy. The only commercially available photosensitizing agent is Photofrin™, a hemato porphyrin or HPD, sold by QLT Phototherapeutics, Inc. Vancouver, British Columbia. Synthetic porphyrins often have the disadvantage of having longer half-lives and lowered sensitivity to the rapidly growing cells as contrasted with normal cells than do naturally occurring porphyrins. The half-life of the photosensitizing agent is significant, since the buildup of excess porphyrins in the skin can result in reddening or burning of the skin.

An alternative to synthetic porphyrins are natural porphyrins. Natural porphyrins appear to have shorter half-lives than their synthetic counterparts, but are difficult to synthesize and more importantly, are unstable ex vivo under environmental conditions to which drugs are subjected in normal commercial distribution and storage channels.

A revolutionary discovery made in the late 1980's was that the naturally occurring amino acid ALA, a precursor in the metabolic pathway to heme, could be used in photodynamic therapy instead of synthetic porphyrins. ALA appears to act in the body as a precursor to naturally occurring, light sensitive porphyrins, which avoids the ex vivo problems associated with natural porphyrins noted above. This discovery has brought photodynamic therapy to a world wide interest level never before achieved with synthetic porphyrins.

ALA has a very short half-life, depending on the route of administration, is highly tissue specific and,

as a naturally occurring amino acid, minimizes complications and side effects which arise when foreign substances are administered to the body. Unlike synthetic porphyrins, ALA also makes it possible to distinguish small or flat tumors, e.g. in the bladder, from normal tissues, visually by means of fluorescence excitation.

It is believed that ALA is converted by the cells and tissues in vivo or ex vivo into protoporphyrin IX and related endogenous biochemicals, which fluoresce or are degraded by light of the appropriate wavelength. The preferential accumulation of such naturally occurring porphyrins in rapidly growing cells permits the targeting of such cells.

One of the roadblocks to the commercial use of ALA has been its extreme liability to destruction under ambient conditions. Aqueous solutions of ALA maintained under ambient conditions are progressively, degraded quite rapidly, resulting in degradation products, primarily 2,5-pyrazine dipropionic acid and intermediate degradation products which have not been able to be identified due to their transient nature. However, the intermediate degradation products are believed to include 2,5 (beta-carboxymethyl)-dihydropyrazine. Figure 1 depicts the degradation of ALA to 2,5 (beta-carboxymethyl)-dihydropyrazine and then to 2,5-pyrazine dipropionic acid. Formulating ALA in nonaqueous creams and gels did not prevent this degradation. Even ALA pressure-sensitive adhesive mixtures did not totally stop oxidative reactions. Likewise, the preparation of pharmacological equivalents of ALA such as functional derivatives of the carboxylic acid group, substitution of the amino group, blocking of the oxo group or the use of simple or more complex acid addition, acid, base or neutral salts has not completely overcome this problem because the more stable the product, the greater effect there may be on the metabolism of the product in the body.

Summary of the Invention

One object of the invention is to provide ALA which does not suffer from the problems of the known art, particularly the extreme degradation of ALA. Another object of the invention is to provide sterile stable ALA which is pharmacologically active and can be used in photodynamic therapy and detection. Still another object of the invention is to provide a method for making sterile, stable ALA which does not suffer from the problems of the known art. Yet another object of the invention is to provide a combination of ALA and an endoscope for internal use in a mammal. Still another object of the invention is to provide a method for using ALA in the detection and/or treatment of a condition in a mammal.

The foregoing objects and other objects are achieved according to one aspect of the present invention by colored 5-aminolevulinic acid, preferably 5-aminolevulinic acid HCl. In a preferred embodiment, the color is imparted by irradiation of crystals, preferably gamma radiation. According to another aspect of the invention, there has been provided colored 5-aminolevulinic acid crystals having an F-center point defect in the crystal lattice, where said F-center point defect imparts said color to the crystals.

According to still another aspect of the invention, there has been provided a sterile aqueous ALA solution comprising the colored ALA crystals according to the invention, contained in water. According to yet another aspect of the invention, there has been provided a sterile package comprising colored ALA crystals according to the invention in a sealed sterile container. According to yet another aspect of the invention, there has been provided a method for preparing colored ALA crystals according to the invention, which includes exposing non-irradiated ALA crystals to a radiation source at a dose sufficient to impart a color which is different than any color present in the non-irradiated

crystals. According to still another aspect of the invention, there has been provided a kit for internal and/or external treatment and/or detection of a condition in a mammal, which includes the sterile colored ALA crystals according to the invention and a sterile diluent, preferably water. In one mode of internal treatment and/or detection, the kit optionally includes a catheter and optionally an endoscope.

According to still another aspect of the invention, there has been provided a method of administering 5-aminolevulinic acid in a stable form for internal and/or external mammal administration which comprises the administration of a solution of ALA derived from the colored ALA according to invention.

Further objects, features and advantages of the present invention will become apparent from consideration of the detailed preferred embodiments which follow.

Brief Description of the Drawings

Figures 1-12 compare gamma irradiated with nonirradiated crystalline ALA. In the figures, "REF" denotes nonirradiated ALA and "IRR" denotes irradiated ALA.

Fig. 1 depicts the degradation reaction pathway of ALA.

Fig. 2 compares thermogravimetic measurements.

Fig. 3. compares differential scanning calorimetry measurements.

Fig. 4 compares reflectance spectra of solid samples.

Fig. 5 compares the photoacoustic spectroscopy ("PAS") spectra.

Fig. 6 compares the PAS spectra of gamma irradiated ALA as a function of the irradiation dose.

Fig. 7 compares the PAS spectra of a single sample of ALA before and 20 minutes after irradiation with visible light at 410 nm (2mW cm^{-2}) and after a dark period of 10 days.

Fig. 8 compares the reflectance spectra before and 2.2 hours after irradiation with visible light at 410 nm (0.12 mW cm^{-2}) and after a 2 day dark period.

Fig. 9 compares the reflectance spectra before and after dissolution in methanol and evaporation of the solvent of ALA irradiated at 25 kGy.

Fig. 10 compares the reflectance spectra prior to and after dissolution in methanol and evaporation of the solvent for a different set of samples than used in Fig. 9 irradiated at 25 kGy.

Fig. 11 compares the absorbance spectra of both gamma-irradiated and reference ALA in methanol for ALA which was stored at -10°C and at 40°C.

Fig. 12 compares the absorbance spectra of both gamma-irradiated and reference ALA upon the first, and second dissolution in 0.2 M PBS, pH 5 for ALA stored at both -10°C and 40°C.

Fig. 13 shows a photograph taken at 4X magnification of gamma-irradiated colored ALA crystals.

Fig. 14 shows a photograph taken at 4X magnification of non-irradiated ALA crystals.

Detailed Description of Preferred Embodiments

5-Aminolevulinic acid is also known as 5-aminolaevulinic acid, δ-aminolevulinic acid, δ-aminolaevulinic acid and 5-amino-4-oxopentanoic acid. 5-Aminolevulinic acid can be used as the salt, particularly a simple salt and especially the hydrochloride salt. 5-Aminolevulinic acid can also be used in the form of a precursor or product of 5-aminolevulinic acid. 5-Aminolevulinic acid can also be used in its pharmacologically equivalent form, such as an amide or ester. Examples of precursors and products of 5-aminolevulinic acid and pharmacologically equivalent forms of 5-aminolevulinic acid that can be used in the present invention are described in J. Kloek et al., Prodrugs of 5-Aminolevulinic Acid for Photodynamic Therapy, Photochemistry and Photobiology, Vol. 64 No. 6, December 1996, pages 994-1000; WO 95/07077; Q. Peng et

al., Build-Up of Esterified Aminolevulinic-Acid-Derivative-Induced Porphyrin Fluorescence in Normal Mouse Skin, Journal of Photochemistry and Photobiology B: Biology, Vol. 34, No. 1, June 1996; and WO 94/06424, which are all incorporated by reference herein in their entirety. As used herein, all of these compounds, unless other wise noted, are referred to jointly and severally as "ALA."

As used herein, the term "sterilized" refers to a product which has been processed to make it suitable for internal pharmaceutical use.

As used herein, the term "colored" is defined as color that has been induced by irradiation. This is to be distinguished from non-irradiated ALA which is generally white, but in some instances may be off-white, probably due to the presence of varying amounts of degradation products.

As used herein, a "pharmaceutically pure" substance is defined as a substance which is suitable for therapeutic and detection use in humans and other mammals. Preferably, the pharmaceutically pure substance is labeled for therapeutic and detection use in humans and other mammals.

The present invention is based on the finding that sterile ALA can be made and prepared well in advance of its final use, despite the extreme degradation generally found with ALA as noted above. It was further and surprisingly found that despite the yellow color of irradiated crystalline ALA, the substance had not been degraded, despite the damaging effects that radiation, particularly gamma radiation, can have on thermodynamically unstable substances. In addition, it has been surprisingly found that the gamma irradiated material is stable for extended periods of time, at least one year, when stored in the sealed bottle in which it was irradiated.

The sterilized colored ALA crystals are preferably substantially free of impurities, such as degradation products and pyrogens, especially when intended for

systemic administration. The amount of impurities is generally ≤ 2.0 wt%, preferably ≤ 1.0 wt%, and more preferably ≤ 0.5 wt%.

The crystalline ALA is colored to the naked eye by irradiation from a source which emits electromagnetic radiation, preferably gamma radiation or other ionizing radiation. For example when the hydrochloride salt of ALA is employed, the irradiation alters the appearance of the crystals to the naked eye from white or off-white to yellow. The intensity of the color, such as the yellow color for ALA HCl, is dependent on the crystalline form that is irradiated, such as the form as commercially supplied or a crystal that is further micronized. For example, when the ALA crystals are micronized, the irradiated ALA micronized crystals are less intensely colored than intact (i.e., non-micronized) ALA crystals. This is consistent with the F-center theory for colorization described below. The intensity is also dependent on the dosage of radiation that the crystalline ALA has been supplied with.

This color disappears after dissolution of the irradiated crystals and does not appear again upon recrystallization from an aqueous solution under ambient (i.e., room temperature and pressure) conditions. The colored crystalline irradiated ALA has not been distinguished in any chemical property from non-irradiated ALA, either by spectroscopic, chromatographic, solution pH or solubility profile, except for the color of the irradiated material. This is explained more fully below with respect to Figs. 2 to 14.

Gamma irradiated ALA, as can be seen from Figs. 2 through 14 is substantially physically and chemically the same, except for the color, as the non-irradiated ALA with respect to spectroscopic, chromatographic, solution pH and solubility profile. Although not intending to be bound by any theory, it appears that the color imparted by irradiation is due to F-centers.

The calorimetric measurements of Figs. 2 and 3 indicate that any major structural differences are

present in quantities of less than 1% of the total ALA amount, or that the type of any structural modification is such that the sensitivity of the calorimetric assay to the change is below the capacity of the instrumentation.

Spectral reflectance as shown in Figures 4 and 8-10 is very sensitive to spectral differences at or near the surface, while photoacoustic spectroscopy ("PAS") as shown in Figures 5-7 is sensitive to changes both at the surface and the interior of the crystal. Both of these techniques revealed significant spectral differences between the irradiated and nonirradiated reference samples of ALA in the solid crystalline phase. Since no differences were observed in the solution phase spectra, the yellow color of the irradiated material is likely to be a property of the crystalline solid only. Calorimetry failed to show gross crystal modifications, supporting the view that the crystal modifications are such that the overall crystal symmetry is not significantly affected.

Although not wishing to be bound by any theory, gamma radiation is known to cause point defects in substances such as alkali halides. Such alterations do not change the overall symmetry of the crystal, since they involve only the removal or relocation of small numbers of specific ions, while essentially leaving the major crystalline structure intact. The most common type of point defect caused by the ionizing radiation of the F-(farben)-center, which is a negative ion vacancy with one excess electron bound at the vacancy. The time required to fill the vacancy formed by the electrons is on the order of minutes to days, and is dependent on the diffusion rate of electrons in the crystal lattice. F-centers have been extensively studied and are characterized by one or more absorbance band(s) at higher wavelengths (lower energy) than that of the normal absorbance of the molecules surrounding the excess electron. These allowed, red-shifted bands are believed to stem from the excess electron gaining state function properties from the surrounding molecules in a symmetrical manner. The location of the UV-visible

absorbance bands seen in Figures 4, 5 and 6, detected by both PAS and reflectance spectroscopy, is consistent with the F-centers.

The spectra shown in the photobleaching studies of Figs. 7 and 8 is consistent with this F-center theory. The spectra depicted in Figs. 9 through 12, suggest that the color is due to an F-center defect rather than preformed chemical degradation. Figures 13 and 14 indicate that no microscopic changes take place upon irradiation of the ALA.

Moreover, when ALA is irradiated, there is generally some delay before coloring of the crystal sets in. As noted above, the coloring is due to an ion being "kicked out" during irradiation, leaving a charged hole. The temporal delay in coloring is believed due to the basis of ion migration through the crystal lattice. This delay further supports the F-center theory.

Although the above measurements and analysis were carried out on ALA HCl, which produces a yellow color, it is fully expected that other crystalline forms of ALA would also experience non-degradation related coloring upon irradiation.

Another aspect of the invention provides methods for the preparation of sterile ALA suitable for internal use in human subjects and other mammals, namely by irradiating, particularly γ-irradiation, of ALA. Preferably, the irradiation is carried out in a sealed container, such that both the ALA and the container are sterilized during irradiation. The irradiation procedure sterilizes the ALA in the conventional sense. Sterilization by radiation, particularly gamma radiation is well known in the art and will not be discussed at length. More detailed information can be found in <u>Gamma Processing Technology: An Alternative Technology for Terminal Sterilization of Parenterals</u>, by B.D. Reid, PDA Journal of Pharmaceutical Science & Technology, vol. 49, no. 2, March-April 1995, pages 83-89, which is incorporated herein in its entirety.

The ALA is irradiated with a dose of radiation sufficient for sterilization. A sufficient dose of radiation can be determined by methods known to those skilled in the art. For example, after irradiating ALA with a selected dose, the ALA can be transferred to the appropriate media for encouraging the growth of viable microorganisms to determine if sterilization is sufficiently complete.

For most applications using gamma irradiation, a dose of 5 kilograys or greater has been found to provide sufficient sterilization. However, as noted above, the crystalline ALA has been surprisingly found to resist degradation even at high doses of gamma radiation. Thus, it is possible to provide a dose of 25 kilograys or greater, without detectable degradation of the ALA. This is significant, in that the United States Food and Drug Administration's ("FDA") level of gamma irradiation for "overkill" is 25 kilograys. This is a level at which the FDA presumes, without evidence, that virtually all microorganisms have been killed.

The present invention also provides for methods of using the sterilized ALA in photodynamic therapy internally or externally on the tissues or cells of a mammal. For external use, the sterilized ALA may be applied by an applicator. For internal use, application may be orally, intravenously or administration by a catheter. For example, the administration of the ALA can be on internal surfaces of the body of the subject, typically in conjunction with an endoscope coupled to a light source. Numerous publications discuss photodynamic therapy, see e.g. Kriegmair et al., "Photodynamic Diagnosis (PDD) for Early Recognition of Carcinomata of the Bladder", ENDO World Uro No. 17-E, 1955, a publication of Karl Storz Gmbh & Co. and the equipment referred to therein. See also:

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of the endogenous photosensitizer protoporphyrin IX following exposure to 5-aminolevulinic acid. Photochem. Photobiol. 1995;62:887-895.

All of the above references are incorporated herein in their entireties. Other photodynamic therapy or photodetection uses include treatment of actinic keratoses, hair removal, treatment of acne and endometrial ablation.

Endoscopes coupled to a light source for use in photodynamic therapy are sold commercially but can be especially designed for use with ALA and its precursors. Suitable endoscopes for use with a light source are commercially available, e.g. from Karl Storz Gmbh & Co., Tuttligen, Germany; Circon ACMI; Olympus; and Richard Wolf. Another suitable endoscope is described in U.S. Patent No. 5,441,531, incorporated herein by reference in its entirety.

This invention also provides for commercial kits containing sterilized ALA, a sterile diluent such as an aqueous buffer solution, and, optionally a catheter for administering the ALA solution. An endoscope coupled to a light source such as those described above, for use in detection or treatment of the cells or tissues in which the ALA preferentially accumulates can also be included in the kit. Preferably instructions on the use of the ALA are packaged with the kits.

Reference will now be made to the following non-limiting examples.

EXAMPLES 1-4 and COMPARATIVE EX. 1

ALA hydrochloride salt was obtained from Sochinaz, S.A., Vionnez, Switzerland. 1.65 grams of ALA hydrochloride salt was placed into 60 ml glass vials. 360 vials of crystalline non-irradiated ALA were prepared. Of these, 225 vials were sealed under ambient conditions,and were irradiated with gamma radiation as shown in Table 1.

Table 1

Example	no. of vials	conditions	target dose (KGys)	color
1	45	crystalline, atmospheric, room	15	yellow
2	45	same as Ex. 1	30	yellow
3	45	crystalline, atmospheric, packed in dry ice	15	yellow
4	45	same as Ex. 3	30	yellow
Comp. Ex. 1	45	same as Ex. 1	0	off white

EXAMPLES 5-6 AND COMPARATIVE EX. 2

The other 135 vials as prepared above were sealed under an argon atmosphere and were irradiated as shown in Table 2.

Table 2

Example	no. of vials	conditions	target dose (KGys)	color
5	45	crystalline, argon atmosphere, room temperature	15	yellow
6	45	same as Ex. 5	30	yellow
Comp. Ex. 2	45	same as Ex. 5	0	off white

EXAMPLES 7-10 AND COMPARATIVE EXS. 3 and 4

Crystalline ALA was first micronized by pulverizing into fine particles a few μm in diameter by a jet mill by Micro-Macinazione, S.A. to determine if further reducing the crystalline size would have any effect on the color of the irradiated ALA. After micronization, 1.65 grams of ALA hydrochloride salt was placed into 60 ml glass vials. A total of 270 vials of crystalline non-irradiated ALA were prepared. Of these, 135 vials were

sealed under ambient conditions and irradiated with gamma radiation as shown in Table 3. The other 135 vials were sealed under an argon atmosphere and irradiated with gamma radiation as shown in Table 3.

Table 3

Example	no. of vials	conditions	target dose (KGys)	color
7	45	micronized crystalline, atmospheric, room temperature	15	yellow
8	45	micronized crystalline, atmospheric, room temperature	30	yellow
Comp. Ex. 3	45	micronized crystalline, atmospheric, room temperature	0	off white
9	45	micronized crystalline, argon, room temperature	15	yellow
10		micronized crystalline, argon, room temperature	30	yellow
Comp. Ex. 4		micronized crystalline, argon, room temperature	0	off white

Subsequent analysis of the type described above with respect to Figures 2-14, confirmed that there was no discernible difference in structure or pharmacological activity between the irradiated examples and the non-irradiated control comparative examples.

The gamma irradiation also results in a darkening of the glass bottle used to contain the ALA. Presumably the

darkening is due to impurities in the glass that do not affect its pharmaceutical acceptability. In fact, this darkening is an advantage since it shades the bottle contents from direct light.

Other embodiments of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. It is intended that the specification be considered as exemplary only, with the true scope and spirit of the invention being indicated by the following claims.

What Is Claimed Is:

1. Colored 5-aminolevulinic acid crystals.

2. The colored 5-aminolevulinic acid crystals according to claim 1, wherein said color is imparted by irradiation of the crystals.

3. The colored ALA crystals according to claim 1, wherein said crystals are pharmaceutically pure and sterile.

4. The colored ALA crystals according to claim 2, wherein said irradiation is gamma irradiation.

5. The colored ALA crystals according to claim 4, wherein said gamma irradiation is sufficient to sterilize said crystals.

6. The colored ALA crystals according to claim 5, wherein said gamma irradiation provides a dose of at least 5 kilograys.

7. The colored ALA crystals according to claim 5, wherein said gamma irradiation provides a dose of at least 25 kilograys.

8. The colored ALA crystals according to claim 1, wherein said ALA contains less than 2.0 wt% impurities.

9. The colored ALA crystals according to claim 1, wherein said ALA contains less than 1.0 wt% impurities.

10. The colored ALA crystals according to claim 1, wherein said ALA contains less than 0.5 wt% impurities.

11. The colored ALA crystals according to claim 2, wherein said crystals dissolved in an aqueous solution have substantially the same spectroscopic,

chromatographic, solution pH and solubility profile as nonirradiated 5-aminolevulinic acid.

12. A sterile ALA solution comprising the colored ALA crystals according to claim 3, contained in a diluent.

13. A sterile package comprising colored ALA crystals according to claim 3 in a sealed sterile container.

14. A sterile package according to claim 13, wherein the container is sterilized by irradiation.

15. A method for preparing colored ALA crystals according to claim 2, comprising exposing non-irradiated ALA crystals to a radiation source at a dose sufficient to impart a color which is different than any color present in the non-irradiated crystals.

16. A method for preparing colored ALA crystals according to claim 15, further comprising: placing non-irradiated ALA crystals into a container; sealing said container; and exposing the non-irradiated ALA crystals and the container to a radiation source at a dose sufficient to impart a color which is different than any color present in the non-irradiated crystals and is sufficient to sterilize said crystals and container.

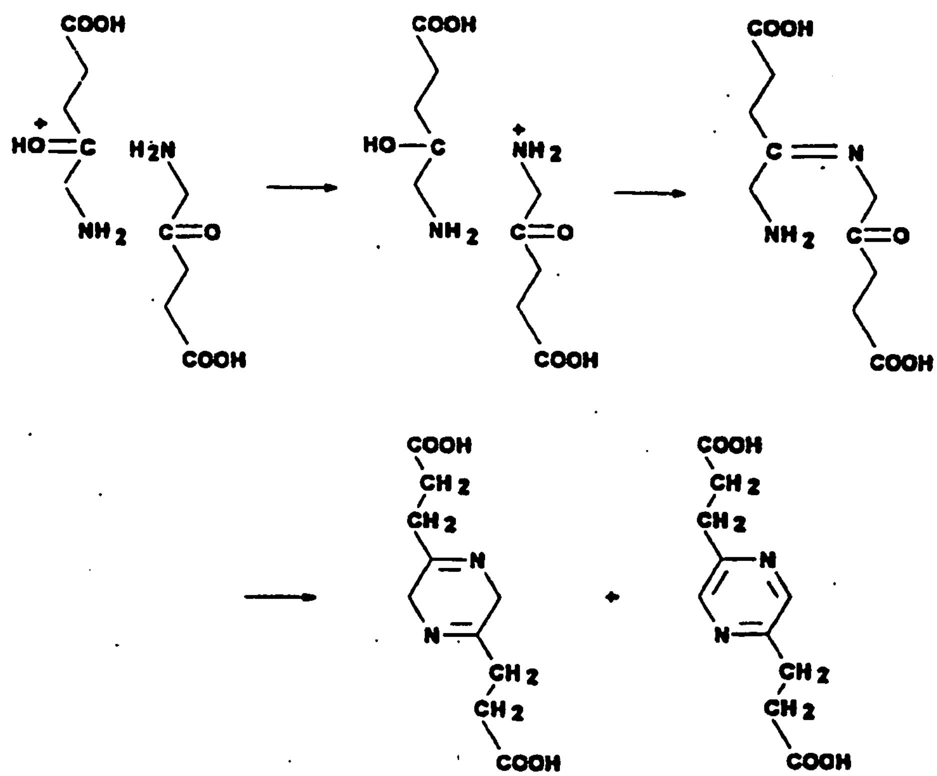
17. A kit for internal treatment and/or detection of a condition in a mammal, comprising the sterile colored ALA crystals according to claim 3 and sterile diluent.

18. A kit according to claim 17, further comprising a catheter.

19. A kit according to claim 17, wherein the colored ALA crystals are packaged in a sterile container.

20. A method of administering 5-aminolevulinic acid in a stable form for internal and/or external mammal administration which comprises the administration of a solution of ALA derived from the colored ALA according to claim 1.

21. Colored 5-aminolevulinic acid crystals comprising an F-center point defect in the crystal lattice, wherein said F-center point defect imparts said color to the crystals.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H2N
NH2
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COOH
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HO—C
NH2
NH2
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NH2
C=O
COOH
COOH
CH2
CH2
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N
CH2
CH2
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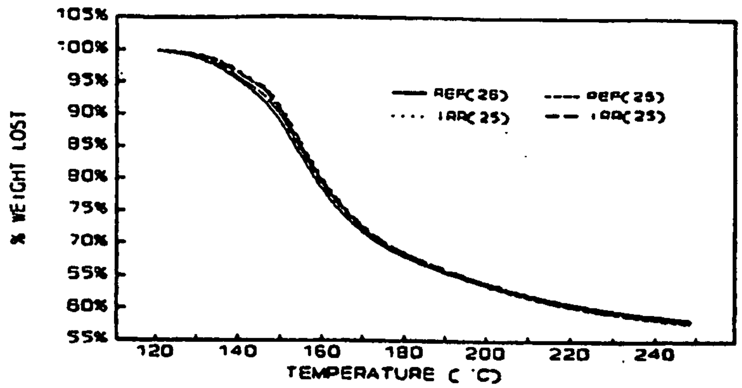
CH2
CH2
N
N
CH2
CH2
COOH

FIG. 1


105%
100%
95%
90%
85%
80%
75%
70%
65%
60%
55%
% WEIGHT LOST
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140
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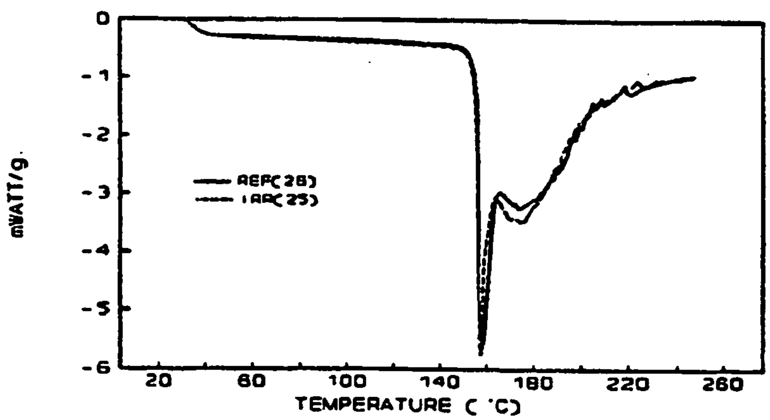
200
220
240
TEMPERATURE (°C)
REF(26)
REF(25)
IAR(25)
IAR(25)

FIG. 2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-3
-4
-5
-6
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FIG. 3

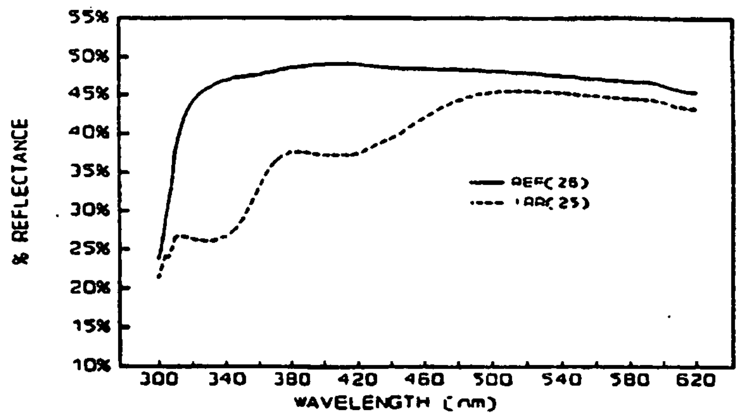

FIG. 4

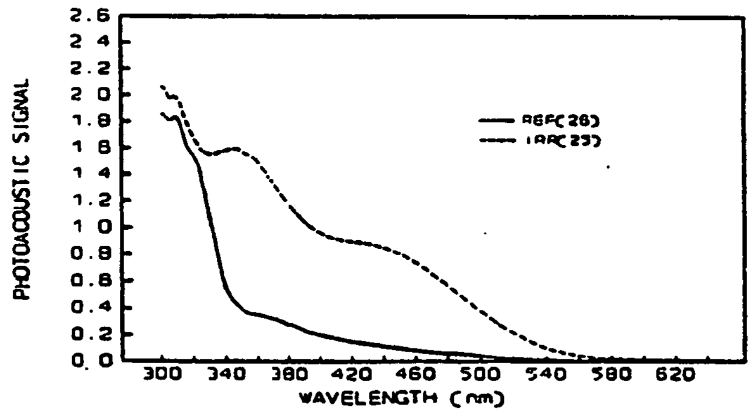

FIG. 5

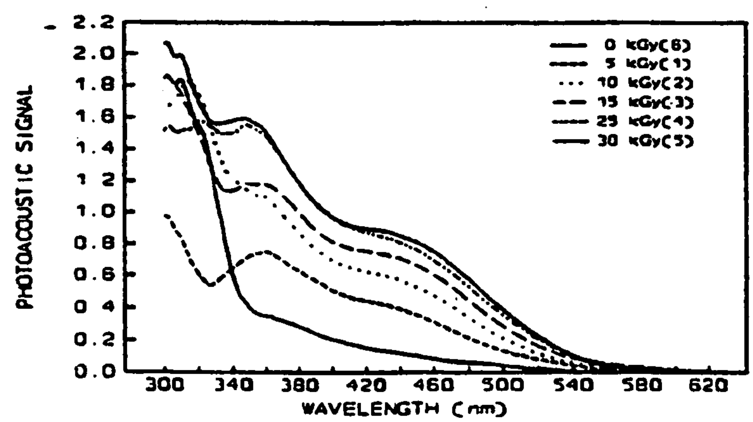

FIG. 6

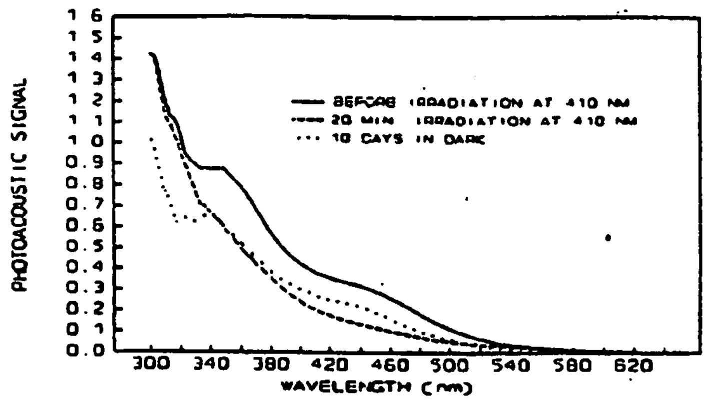

FIG. 7

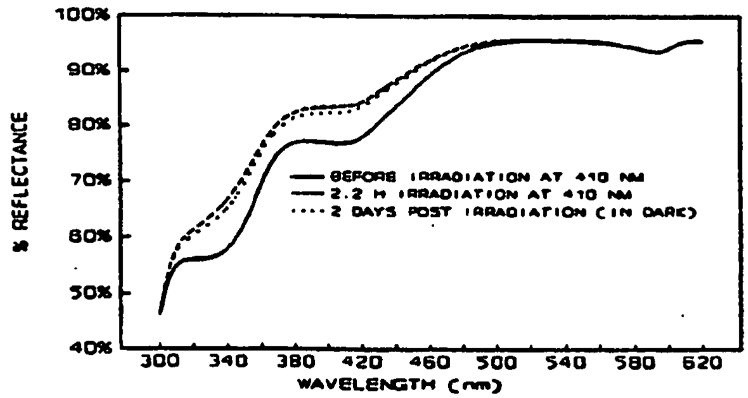

FIG. 8

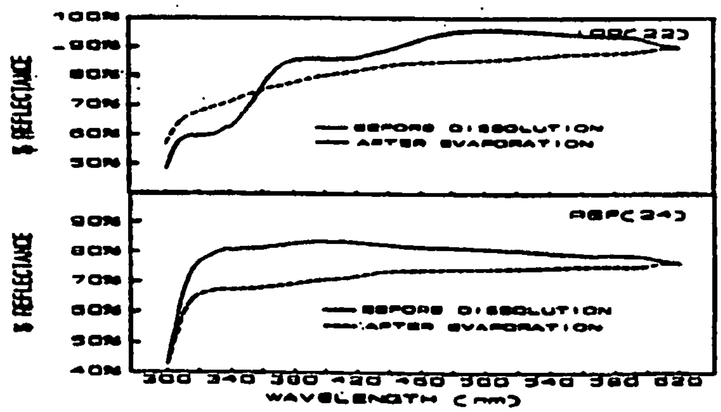

FIG. 9

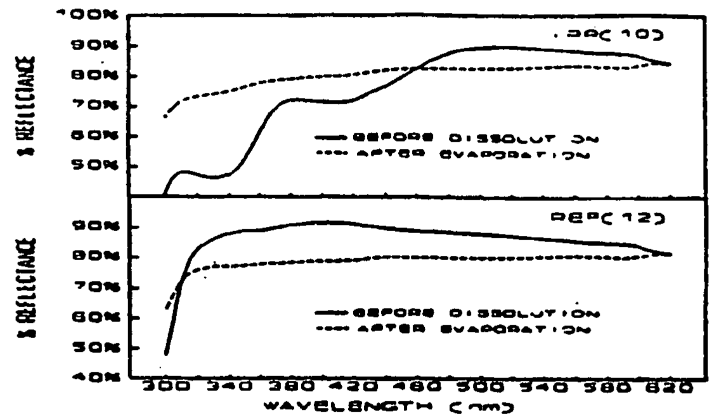

FIG. 10

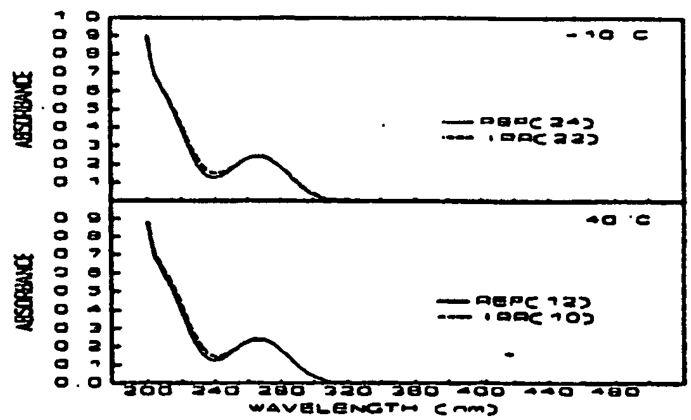

FIG. 11

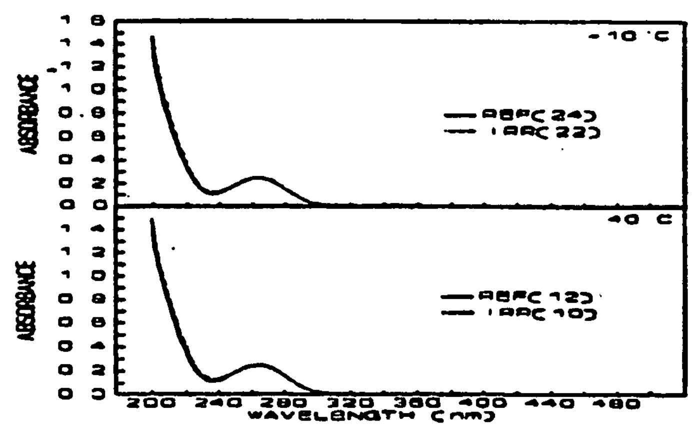

FIG. 12

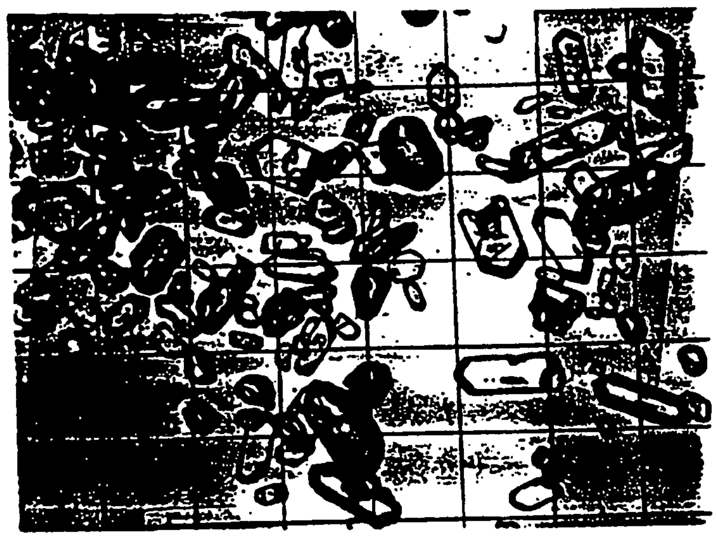

FIG. 13

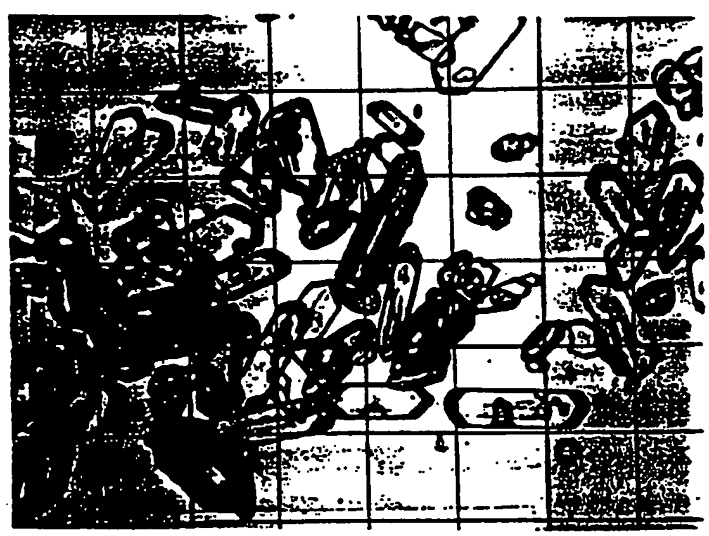

FIG. 14

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/18181

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07C229/22 C07C227/42 A61K51/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07C A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE WPI Section Ch, Week 9545 Derwent Publications Ltd., London, GB; Class B05, AN 95-348331 XP002087678 & JP 07 238063 A (COSMO OIL CO LTD) , 12 September 1995 see abstract ---	1-11
A	WO 94 06424 A (QUADRA LOGIC TECH INC) 31 March 1994 cited in the application see the whole document --- -/--	1-20

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 December 1998	04/01/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Rufet, J

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Form PCT/ISA/210 (second sheet) (July 1992)

Inter	onal Application No
PCT/US 98/18181

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	WO 95 07077 A (NORWEGIAN RADIUM HOSPITAL RESE ;DZIEGLEWSKA HANNA EVA (GB); GIERSK) 16 March 1995 cited in the application see the whole document ---	1-20
A	PATENT ABSTRACTS OF JAPAN vol. 011, no. 326 (C-454), 23 October 1987 & JP 62 111954 A (JAPAN SPECTROSCOPIC CO), 22 May 1987 see abstract ---	1,20
A	BRIAN D. REID: "Gamma processing technology: An alternative technology for terminal sterilization of Parenterals" JOURNAL OF PHARMACEUTICAL SCIENCE & TECHNOLOGY , vol. 49, no. 2, 1995, pages 83-89, XP002087677 cited in the application see the whole document -----	15,16

1

INTERNATIONAL SEARCH REPORT

.iformation on patent family members

Inter nal Application No
PCT/US 98/18181

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